

SQUIRRELL et al.
Appl. No. 09/529,722
February 14, 2005

IN THE CLAIMS:

Amend the claims as follows.

Claims 1-106. (Canceled)

107. (New) A method for producing a luciferase which is substantially free of *E. coli* adenylate kinase, the method comprising culturing an *E. coli* host cell which has been transformed so that it expresses a luciferase which is thermostable at 37°C, and expresses adenylate kinase only in a mutant form which form is denatured at temperatures of 37°C; and recovering the luciferase, wherein either the host cell culture or the recovered luciferase is subjected for a sufficient period of time to a temperature at which the adenylate kinase is denatured but the luciferase remains substantially unaffected, so as to denature the adenylate kinase.

108. (New) A method according to claim 107 wherein the luciferase is a luciferase selected from the group consisting of Photinus pyralis luciferase which has a mutation at position 354 in the amino acid sequence, or a *Luciola* luciferase with a mutation at position 354, which mutation elevates the thermostability of the protein over that of the wild-type protein.

109. (New) A method according to claim 107 wherein the luciferase is selected from the group consisting of *Luciola* luciferase in which the amino acid at the 217 position is mutated to a hydrophobic amino acid.

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110. (New) A method according to claim 107 wherein the *E. coli* host cells are cultured for a period which is sufficient to allow production of the luciferase, and then a batch of said culture is subjected to a temperature at which the adenylate kinase is denatured, and the luciferase is recovered from the said batch.

111. (New) A method according to claim 107 wherein the adenylate kinase includes mutations at amino acids 87 or 107 in the sequence of *E. coli* adenylate kinase.

112. (New) A method according to claim 106 wherein the said temperature is a temperature of from 37°C up to the temperature at which the luciferase is denatured.

113. (New) A recombinant *E. coli* cell which has been transformed so that it expresses a first nucleotide sequence which encodes a luciferase which is stable at 37°C under the control of regulatory elements which allow expression of said polypeptide, and is further transformed so that it expresses adenylate kinase only in a mutated form which is denatured at 37°C.

114. (New) A recombinant cell according to claim 113 which further comprises at least one selection marker.

115. (New) A recombinant cell according to claim 113 wherein the luciferase is a Photinus pyralis luciferase which has a mutation at position 354 in the amino acid

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sequence, or a *Luciola* luciferase with a mutation at position 354, which mutation elevates the thermostability of the protein over that of the wild-type protein.

116. (New) A recombinant cell according to claim 113 wherein the luciferase is a *Luciola* luciferase in which the amino acid at the 217 position is mutated to a hydrophobic amino acid.

117. (New) A method for producing a recombinant cell according to claim 113 which method comprises in any order (a) transforming a host cell with a vector which encodes adenylate kinase in a form which is denatured at 37°C, subjecting transformants to said conditions and detecting those in which protein product is denatured, and (b) transforming said host cell with a vector which encodes the said luciferase and a first selection marker, and using the first selection marker to detect stable transformants.

118. (New) A method according to claim 117 wherein the vector which encodes said adenylate kinase further comprises a second selection marker which is different to said first selection marker, and stable transformants are selected.

119. (New) A method according to claim 116 wherein said selection markers comprise particular different antibiotic resistance genes.

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120. (New) A method for producing a luciferase which is substantially free of *E. coli* adenylate kinase, the method comprising culturing an *E. coli* host cell which has been transformed so that it expresses a luciferase selected from the group consisting of Photinus pyralis luciferase which has a mutation at position 354 in the amino acid sequence, or a *Luciola* luciferase with a mutation at position 354, which mutation elevates the thermostability of the protein over that of the wild-type protein, and expresses adenylate kinase only in a mutant form which has mutations at amino acids 87 or 107 in the sequence of *E. coli* adenylate kinase; and recovering the luciferase, wherein either the host cell culture or the recovered luciferase is subjected for a sufficient period of time to a temperature at which the adenylate kinase is denatured but the luciferase remains substantially unaffected, so as to denature the adenylate kinase.

121. (New) A method according to claim 120 wherein the *E. coli* host cells are cultured for a period which is sufficient to allow production of the luciferase, and then a batch of said culture is subjected to a temperature at which the adenylate kinase is denatured, and the luciferase is recovered from the said batch.

122. (New) A method according to claim 121 wherein the said temperature is a temperature of from 37°C up to the temperature at which the luciferase is denatured.

123. (New) A recombinant *E. coli* cell which has been transformed so that it expresses a luciferase selected from the group consisting of Photinus pyralis luciferase which has a mutation at position 354 in the amino acid sequence, or a *Luciola* luciferase

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with a mutation at position 354, which mutation elevates the thermostability of the protein over that of the wild-type protein, under the control of regulatory elements which allow expression of said polypeptide, and is further transformed so that it expresses adenylate kinase only in a mutated form which has mutations at amino acids 87 or 107 in the sequence of *E. coli* adenylate kinase.

124. (New) A recombinant cell according to claim 123 which further comprises at least one selection marker.

125. (New) A method for producing a recombinant cell according to claim 123 which method comprises in any order (a) transforming a host cell with a vector which encodes adenylate kinase in a form which has mutations at amino acids 87 or 107 in the sequence of *E. coli* adenylate kinase, subjecting transformants to said conditions and detecting those in which protein product is denatured, and (b) transforming said host cell with a vector which encodes the said luciferase and a first selection marker, and using the first selection marker to detect stable transformants.

126. (New) A method according to claim 125 wherein the vector which encodes said adenylate kinase further comprises a second selection marker which is different to said first selection marker, and stable transformants are selected.

127. (New) A method according to claim 126 wherein said selection markers comprise particular different antibiotic resistance genes.

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128. (New) A method for producing a luciferase which is substantially free of *E. coli* adenylate kinase, the method comprising culturing an *E. coli* host cell which has been transformed so that it expresses a *Luciola* luciferase in which the amino acid at the 217 position is mutated to a hydrophobic amino acid, and expresses adenylate kinase and expresses adenylate kinase only in a mutant form which has mutations at amino acids 87 or 107 in the sequence of *E. coli* adenylate kinase; and recovering the luciferase, wherein either the host cell culture or the recovered luciferase is subjected for a sufficient period of time to a temperature at which the adenylate kinase is denatured but the luciferase remains substantially unaffected, so as to denature the adenylate kinase.

129. (New) A method according to claim 128 wherein the *E. coli* host cells are cultured for a period which is sufficient to allow production of the luciferase, and then a batch of said culture is subjected to a temperature at which the adenylate kinase is denatured, and the luciferase is recovered from the said batch.

130. (New) A method according to claim 128 wherein the said temperature is a temperature of from 37°C up to the temperature at which the luciferase is denatured.

131. (New) A recombinant *E. coli* cell which has been transformed so that it expresses a *Luciola* luciferase in which the amino acid at the 217 position is mutated to a hydrophobic amino acid, under the control of regulatory elements which allow expression of said polypeptide, and is further transformed so that it expresses adenylate

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kinase only in a mutated form and expresses adenylate kinase only in a mutant form which has mutations at amino acids 87 or 107 in the sequence of *E. coli* adenylate kinase.

132. (New) A recombinant cell according to claim 131 which further comprises at least one selection marker.

133. (New) A method for producing a recombinant cell according to claim 131 which method comprises in any order (a) transforming a host cell with a vector which encodes adenylate kinase in a form which has mutations at amino acids 87 or 107 in the sequence of *E. coli* adenylate kinase, subjecting transformants to said conditions and detecting those in which protein product is denatured, and (b) transforming said host cell with a vector which encodes the said luciferase and a first selection marker, and using the first selection marker to detect stable transformants.

134. (New) A method according to claim 131 wherein the vector which encodes said adenylate kinase further comprises a second selection marker which is different to said first selection marker, and stable transformants are selected.

135. (New) A method according to claim 134 wherein said selection markers comprise particular different antibiotic resistance genes.